The Enthalpy of Self-Association of Purine Derivatives in Water*

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ABSTRACT: The relative molal enthalpies of a series of purine compounds (purine, purine riboside, deoxyadenosine, and caffeine) have been measured over a range of concentrations approaching the solubility limit in water at 25°. These measurements, when used in conjunction with the osmotic coefficient data of other workers, enable the evaluation of the enthalpy of self-association for these materials. The values of ΔH° range from -2 to -4.2 kcal/mole. From measured equilibrium constants and ΔH° values it is found that significantly different entropies of association are found for this series of compounds. The water solvent plays a complicated role in the self-association of these materials. A substitution of a methyl at the 6 position of the purine moiety produces a decrease of the enthalpy of interaction by approximately 2 kcal/mole. This result is in excellent agreement with quantum theoretical calculations on purine and 6-methylpurine.

The use of model compounds, which are closely related to constituents of nucleic acid, provides a means for investigating the molecular features that contribute to the structural stability of nucleic acid. Thermodynamic studies of the self-association of a number of purine and pyrmidine compounds (Solie, 1965; Ts'o *et al.*, 1963; Ts'o and Chan, 1964; Guttman and Higuchi, 1957) have shown that purine compounds tend to form complexes of greater stability than the pyrimidine compounds.

In the investigation of enthalpy and entropy contributions to the free energy, calorimetric measurements provide the most direct method for the accurate determination of the enthalpy of the reaction. When used in conjunction with equilibrium constants or osmotic coefficient data (Schellman, 1955), heats of infinite dilution can be interpreted in terms of the enthalpy of reaction. Under circumstances where the association is characterized by dimerization or by multiequilibria with equal enthalpy and free energy, both the equilibrium constant and the enthalpy of reaction can be obtained from heats of dilution data (Stoesser, 1966). The stacked complex of 6-methylpurine was found by this means to have a large enthalpy of self-association of -6.0 ± 0.4 kcal mole^{-1} (Stoesser, 1966).

In this paper we present calorimetric findings on a series of purine compounds for which osmotic coefficient or equilibrium constant data are available. The purpose of the calorimetric determinations was to find (a) whether significant enthalpy differences occur in the association of various purine derivatives, and (b) the magnitude of entropy effects in the formation of purine complexes.

Materials and Methods

The deoxyadenosine was obtained from California Biochemicals. Purine and purine riboside (grade I) were obtained from Cyclo Chemical Corp. The purity, as checked by paper chromatography, was estimated to be at least 99%. Caffeine, USP grade, was recrystallized twice from water and sublimed. The compounds were dried under vacuum for 16 hr prior to preparation of solutions.

A special mixing microcalorimeter (Stoesser, 1966) was used in carrying out the dilution measurements. Solutions, prepared from doubly distilled water, were diluted with various amounts of water within the calorimeter. The heat measurements in all cases were reproducible to within 1%. A small correction, less than 5%, was made for the mechanical effects of mixing solution with solvent. The accuracy of the heat measurements was confirmed by diluting a standard solution of urea with water. The measured heat agreed to within 1% of that determined by detailed studies of urea solutions (Gucker and Pickard, 1940).

Theory

Heat of dilution and osmotic coefficient data have been used (Schellman, 1955) to yield the enthalpy of reaction for simple self-association processes. In general one assumes that all heat of dilution effects are due to the dissociation of various complex species of the form A_n, related by the typical equilibria

$$A + A_{n-1} = A_n \qquad (1)$$

with an equilibrium constant K_n and an enthalpy of reaction ΔH_n°. The heat of infinite dilution of an m molal solution may be expressed in terms of the relative molal enthalpy (φ_L) by $-m\varphi_L$ and gives the amount

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of heat due to the dilution of m moles of solute of an m molal solution to a state of infinite dilution. This heat is equal to the effect of dissociating the various species described by eq 1 and is given by

$$m\varphi_L = (A_2)\Delta H_2^\circ + (A_3)(\Delta H_2^\circ + \Delta H_3^\circ) + \ldots \quad (2)$$

where (A_n) represents the molal concentration of species A_n. In the case of dimerization, only the first term would be present. For many situations one might suppose that the enthalpies of the various reaction steps would be quite similar and for such a case

$$\Delta H_2^\circ = \Delta H_3^\circ = \ldots \Delta H^\circ \quad (3)$$

so that

$$m\varphi_L = \Delta H^\circ[(A_2) + 2(A_3) + 3(A_4) + \ldots] \quad (4)$$

The species concentration terms of the last expression bear a simple relation to the experimentally determined osmotic coefficient, φ (Ts'o and Chan, 1964).

$$\varphi = [(A) + (A_2) + \ldots]/m \quad (5)$$

Since the solution molality m is given by

$$m = (A) + 2(A_2) + 3(A_3) + \ldots \quad (6)$$

it can be seen that

$$m(1 - \varphi) = (A_2) + 2(A_3) + \ldots \quad (7)$$

Therefore when there are multiple species with equal enthalpies of reaction (eq 1) and the various species are assumed to each behave ideally, one obtains the general result from eq 4 and 7 that

$$\Delta H^\circ = \varphi_L/[m(1 - \varphi)] \quad (8)$$

For complicated cases this equation would apply to the initial dimerization process upon extrapolation to infinite dilution. In general it is valid irrespective of the equilibrium constants of eq 1 provided the enthalpies of forming each species present obey a simple equality described by eq 3. From a study of the concentration dependence of the osmotic coefficient it is possible in simple situations (Ts'o and Chan, 1964) to evaluate the appropriate equilibrium constants for equilibria 1.

An alternate procedure for evaluating both the equilibrium constant and enthalpy of reaction (Stoesser, 1966) can be used from a study of the concentration dependence of the relative molal enthalpy. The application of this technique depends upon knowledge of the type of reaction (dimerization, or equal-step multiple-species reactions). In spite of this limitation the calorimetric data can be obtained with greater accuracy at lower concentrations than the measurements of osmotic coefficients. For the case of multiple species with equal equilibrium constants K and equal enthalpies of reaction as given by eq 3 one finds the relation (Stoesser, 1966)

$$\varphi_L = \Delta H^\circ - \left(\frac{\Delta H^\circ}{K}\right)^{1/2}(\varphi_L/m)^{1/2} \quad (9)$$

The application of this equation enables the determination of both ΔH° and K.

Results

In all cases the heat effect upon dilution was found to be endothermic. The heat per mole of solute for a given dilution process was determined for the various compounds. It was possible to obtain particularly accurate values over a wide range of concentration for purine due to its great solubility. A plot of the heat per mole *vs.* the final concentration upon dilution was used to extrapolate to a value of the heat of infinite dilution per mole (φ_L) from a given initial concentration. Once

TABLE I: Relative Molal Enthalpy of Aqueous Purine Solutions at 24.9°.

m	$-\varphi_L$ (cal/mole)	φ (osmotic coefficient)[a]	ΔH° (eq 8) (kcal/mole)
1.000	2100 ± 60	0.505	−4.2 ± 0.1
0.480	1660 ± 67	0.609	−4.2 ± 0.1
0.315	1373 ± 67	0.678	−4.3 ± 0.2
0.1555	942 ± 71	0.789	−4.5 ± 0.3
0.0840	690 ± 80	0.845	−4.5 ± 0.5
0.0578	510 ± 70	0.906	−5.4 ± 0.8
0.0296	340 ± 80		
0.0332	340 ± 80		
0.01652	215 ± 80		
0.0092	145 ± 80		
0.0062	105 ± 80		

[a] Values calculated from polynomial equation given by Ts'o *et al.* (1963).

TABLE II: Relative Molal Enthalpy of Aqueous Deoxyadenosine Solutions at 25.1°.

m	$-\varphi_L$ (cal/mole)	φ (osmotic coefficient)[a]	ΔH° (eq 8) (kcal/mole)
0.0345	900 ± 30	0.76 ± 0.04	−3.7 ± 0.6
0.01725	530 ± 35	0.84 ± 0.07	−3.3 ± 1.4
0.01125	370 ± 35	0.88 ± 0.12	−3.2 ± 3.0
0.00574	165 ± 40		
0.00314	110 ± 40		
0.00216	72 ± 45		
0.00111	25 ± 60		

[a] Values interpolated from data of Solie (1965).

this value was determined then the relative molal enthalpies for all the intermediate concentrations can be evaluated by appropriate subtraction. These results are gathered together in Tables I–IV. Also included in these tables are values of the osmotic coefficients determined by other workers for concentrations comparable to those of the calorimetric determinations.

TABLE III: Relative Molal Enthalpy of Aqueous Purine Riboside Solutions at 24.9°.

m	$-\varphi_L$ (cal/mole)	φ (osmotic coefficient)[a]	$\Delta H°$ (eq 8) (kcal/mole)
0.1496	480 ± 20	0.768 ± 0.010	−2.1 ± 0.1
0.1250	418 ± 21		
0.1000	347 ± 22	0.815 ± 0.015	−1.9 ± 0.2
0.0748	269 ± 24	0.827 ± 0.020	−1.6 ± 0.3
0.0499	178 ± 26	0.870 ± 0.034	−1.4 ± 0.4
0.0259	85 ± 32		
0.0136	48 ± 35		

[a] Solie (1965).

TABLE IV: Relative Molal Enthalpy of Aqueous Caffeine Solutions at 24.8°.

m	$-\varphi_L$ (cal/mole)	φ (osmotic coefficient)	$\Delta H°$ (eq 8) (kcal/mole)
0.1000	1175 ± 25	0.650,[a] 0.645[b]	−3.4 ± 0.1
			−3.3 ± 0.1
0.0666	1000 ± 25	0.703[b]	−3.4 ± 0.1
0.0500	865 ± 25	0.723[b]	−3.1 ± 0.2
0.0333	700 ± 30	0.794[b]	−3.4 ± 0.2
0.0167	499 ± 35	0.870[b]	−3.5 ± 0.2
0.0091	284 ± 35		
0.0063	225 ± 40		
0.0032	104 ± 40		

[a] Value given by Ts'o *et al.* (1963). [b] Values calculated from interpolated equilibrium constants ($K_1 = 13.2$, $K_3 = 2160$) at 25° from data of Guttman and Higuchi (1957). We have assumed their molarities are equivalent to molalities at these low concentrations.

For purine solutions it has been shown (Ts'o *et al.*, 1963) that the mechanism with equal equilibrium constants between successive complex species fits the osmotic coefficient data. Equation 9 is therefore assumed applicable for the analysis of the purine calorimetric data. The values of $\Delta H°$ and K from eq 9 calculated from data in Table I are −3.7 ± 0.2 kcal/mole and −2.9 ± 0.2 m^{-1} as compared to −4.2 kcal/mole and −2.1 m^{-1} using the osmotic data of Ts'o *et al.* (1963). It is not possible to use this method for deoxyadenosine or purine riboside due to the low solubility of these compounds and the resulting uncertainty of the heat measurements.

In the case of caffeine the calorimetric data are sufficiently accurate to apply eq 9 although the justification for doing so is questionable in view of the work of Guttman and Higuchi. They suggest that only monomer, dimer, and tetramer exist in aqueous solutions. The values of $\Delta H°$ and K from the use of eq 9 are −2.6 ± 0.2 kcal/mole and 15 ± 1 m^{-1}. In Table V we have summarized the thermodynamic parameters for the self-association of the compounds mentioned above as well as 6-methylpurine.

The two methods of analysis of the calorimetric data give consistent results for purine and 6-methylpurine. For the case of caffeine the enthalpies calculated by the use of indirect osmotic coefficients give consistent results for a eightfold change in concentration. The application of the second method, eq 9, for caffeine gives a value of $\Delta H°$ (−2.6 ± 0.2 kcal/mole) which appears beyond the range of the possible error in the applicability of eq 9 when compared to the values given by eq 8. Therefore, it appears that a mechanism with equal K values of stepwise equilibria (eq 1) is indeed ruled out for the caffeine case. This result is consistent with the findings of Guttman and Higuchi (1957), although it is difficult to imagine the molecular basis which is responsible for the absence of trimer. It would be of considerable interest to have accurate direct osmotic coefficients for aqueous caffeine solutions.

The greatest uncertainty in the values given for deoxyadenosine and purine riboside are due to the osmotic coefficients. Osmotic coefficient measurements and ultracentrifugation measurements gave similar equilibrium constants (Solie, 1965). However, in some recent work P. O. P. Ts'o (personal communications) has indicated that he finds different values for the osmotic coefficient for deoxyadenosine.

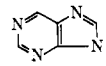
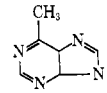
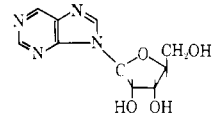
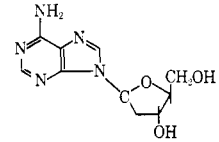
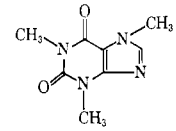
In view of the much lower solubility of these compounds the possibility for error in the osmotic coefficient determinations is greatly amplified. Until more accurate osmotic coefficients are reported for these compounds it will be necessary to recognize the possible uncertainty in their thermodynamic values in Table V.

Discussion

The self-association complexes formed from the compounds listed in Table V are presumably stacked complexes. This conclusion is based on nuclear magnetic resonance studies of purine and 6-methylpurine (Chan *et al.*, 1964), of ultraviolet hypochromic shifts for deoxyadenosine (Solie, 1965), and of nuclear magnetic resonance study of caffeine solutions (A. A. Sandoval and M. W. Hanna, personal communications, 1966). A stacking complex is to be expected for caffeine where the possibility of hydrogen bonding between molecules does not exist.

As seen in Table V the enthalpies of self-association are markedly influenced by structural modification of the purine ring. The free energy of self-association does

TABLE V: Thermodynamic Parameters of Self-Association of Purine Derivatives.

Compound	Structure	$\Delta H°$ (kcal/mole)	$\Delta G°$ (kcal/mole)	$\Delta S°$ (eu)	K (m^{-1})
Purine[e]		-4.2 ± 0.2	-0.44[a]	-13	2.1[a]
6-Methylpurine[e]		-6.0 ± 0.4[b]	-1.12[a]	-16	6.7[a]
Purine riboside[g]		-2.0 ± 0.2	0.5 ± 0.2[c]	-5	2.5 ± 1[c]
Deoxyadenosine[g]		-3.7 ± 0.6	-1.5	-7	12[c]
Caffeine[f]		-3.4 ± 0.2	-1.5	-6	13 ± 1[d]

[a] Ts'o and Chan (1964). [b] Stoesser (1966). [c] Solie (1965). [d] Guttman and Higuchi (1957). [e] Estimation of $\Delta H°$ values for these compounds determined by the two methods described in text agree within experimental error. The limit of errors and the difference between values are therefore considered reliable. [f] The $\Delta H°$ values for caffeine determined by two methods do not agree within experimental error. The value reported here is the largest $-\Delta H°$. The value and the variation assigned rest on the assumption that no trimer is formed. [g] The $\Delta H°$ values reported for these compounds can be calculated by one method only and are highly dependent on experimental determination of osmotic coefficients. Since there is some uncertainty in reported values for osmotic coefficients the error may be larger than indicated.

not correlate well with the changes in enthalpy. Hence, the entropy of self-association plays an important role in the formation of these complexes. The complexity of the entropy effect is most likely a reflection of the changes in the water solvent structure and of the stereochemistry of the interacting species.

A substitution of methyl at the 6 position on the purine ring has a stabilizing effect of from 2.0 to 2.5 kcal on the experimental enthalpy of self-association. It is of interest to note that quantum mechanical calculations on the self-interaction energy of purine and of 6-methylpurine (Pullman *et al.*, 1965; Van de Vorst and Pullman, 1965) show that the methyl substitution causes a stabilization interaction of between 2 and 3 kcal/mole.

The addition of the ribose on purine and deoxyribose on adenine appears to prevent the development of a large enthalpy contribution for these compounds, perhaps due to an effect on the stacking conformation. It is surprising that the enthalpy of association for deoxyadenosine in water is not closer to that found (-8 kcal/mole^{-1}) for stacking interaction in diadenylic acid (Van Holde *et al.*, 1965). Perhaps the more severe steric requirements of diadenylic acid rule out many possible interaction configurations with lower enthalpies that are permitted with deoxyadenosine.

The results with caffeine are also noteworthy. A strong stacking interaction might be expected for this compound. Because the dimerization constant is large one might have expected a large value of the enthalpy of reaction. The finding that only a moderate value (-3.4 kcal/mole) exists again serves to emphasize the importance of the entropy contribution in the formation of these complexes.

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Molecular Weight Dependence of the Rotor Speed Induced Aggregation of Deoxyribonucleic Acid*

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ABSTRACT: The molecular weight dependence of the rotor speed induced aggregation phenomenon has been studied using deoxyribonucleic acids (DNAs) isolated from the T_3, T_4, T_5, and T_7 bacteriophages. A linear relation between the reciprocal of the monomer concentration and the square of the rotor speed has been found in each case. This relation may be termed the pseudo-phase-transition line. Both the slope and the intercept of the pseudo-phase-transition line are functions of the molecular weight of the DNA, and the slope appears to depend upon the three-halves power of the molecular weight. A quantitative theory has been developed in an attempt to explain the behavior of these molecules in the centrifuge.

The anomalous rotor speed dependence of the sedimentation coefficient of high molecular weight DNA isolated from the T-even bacteriophage (Hearst and Vinograd, 1961; Burgi and Hershey, 1961; Crothers and Zimm, 1965; Aten and Cohen, 1965) and bacteria (Eigner *et al.*, 1962) is caused by an aggregation process which is dependent upon the angular velocity of the ultracentrifuge (Rosenbloom and Schumaker, 1963). When such bacteriophage DNA preparations are sedimented in the analytical ultracentrifuge in density gradients to prevent convection, the following events are noted. (1) A variable fraction of the DNA, depending upon the initial concentration and the rotor speed, behaves like a precipitate and sediments rapidly out of solution. (2) The concentration of DNA which remains in solution and sediments normally is inversely proportional to the square of the rotor speed and independent of the initial concentration. (3) The sedimentation coefficient of the DNA remaining in solution is not a function of the rotor speed, but is dependent upon the concentration of DNA present during the remainder of the run.

In order to explain these observations, Rosenbloom and Schumaker (1963) have suggested that the collision rate between small aggregates, formed initially by thermal motion, and single DNA molecules is increased as the sedimentation velocity increases. This increased collision frequency leads to the formation of larger aggregates and ultimately to the separation of a solid phase which precipitates from solution. The DNA remaining in solution sediments normally with a sedimentation coefficient which is independent of rotor speed but markedly dependent upon the concentration of DNA existing during the run. Since the nature of the forces involved in the molecular interaction are probably those of steric entanglement, the precipitation phenomenon should depend upon the molecular weight of the DNA. In order to test this hypothesis we have studied the DNAs isolated from bacteriophage T_5, containing DNA of 84,000,000 molecular weight (Hershey *et al.*, 1962); T_7 and T_3 both having DNA of molecular weights of 25,000,000 (Crothers and Zimm, 1965; Davidson and Freifelder, 1962); and preparations of T_4 DNA sheared to a molecular weight of 65,000,000. We have found that the precipitation

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